Chromanols from *Sargassum siliquastrum* and Their Antioxidant Activity in HT 1080 Cells

Jung Im Lee and Youngwan Seo*

Division of Marine Environment and Bioscience, Korea Maritime University; Busan 606–791, Korea.
Received January 1, 2011; accepted March 2, 2011; published online March 8, 2011

Six meroterpenoids (compounds 1—6) of chromene class, including three known compounds (1—3), were isolated from *Sargassum siliquastrum*. The structure of these compounds was established by extensive 2D-NMR experiments such as ^{1}H gradient double quantum filtered correlation spectroscopy (gDQCOSY), total correlation spectroscopy (TOCSY), nuclear Overhauser effect spectroscopy (NOESY), gradient heteronuclear multiple quantum coherence (gHMQC), and gradient heteronuclear multiple bond correlation (gHMBC), and by comparison with published spectral data. The antioxidant activity of these compounds was evaluated by various antioxidant tests, such as scavenging effects on generation of intracellular reactive oxygen species (ROS), increments of intracellular glutathione (GSH) level, and inhibitory effects on lipid peroxidation in human fibrosarcoma HT 1080 cells. Compounds (1—6) significantly decreased generation of intracellular ROS and inhibited lipid peroxidation while they increased levels of intracellular GSH at a concentration of 5 μg/ml.

Key words *Sargassum siliquastrum*; chromanol; antioxidant activity; intracellular reactive oxygen species

Brown algae of the genus *Sargassum* are widely distributed in the temperate and tropical oceans of the world, and often dominate benthic algal communities, occurring in huge floating masses. Most species have cycles of vegetative growth and attrition.[1)] A number of researchers have reported their biological activities[2—9)] as well as secondary metabolites.[10—23)] The brown alga *S. siliquastrum* is commonly found in the coastal area of the Korean peninsula. Recently, some studies have addressed that *S. siliquastrum* possesses a number of meroditerpenoids of the chromene and related structural class[24—27)] and some of these meroditerpenoids exhibit biological activities such as cytotoxicity, antioxidant capacity, vasodilatation, inducement of the larval settlement of the hydrozoan, and inhibition of butylcholine esterase.[28—30)]

In the course of our countinuing search for bioactive compounds from marine algae, the brown alga *S. siliquastrum* was collected along the offshore of Jeju Island, Korea. Antioxidant activity of its organic extract was evaluated on ROS in cellular system. Bioactivity-guided partitioning and various chromatographic methods resulted in the isolation of several compounds of chromene class. Herein we report structure elucidation of chromanols (**1**—**6**), together with three new metabolites (**4**—**6**), and their scavenging effect on ROS in HT 1080 cells.

Results and Discussion

The brown alga *Sargassum siliquastrum* was collected along the offshore of Jeju Island, Korea. The collected samples were extracted with acetone–CH_2Cl_2 (1 : 1) and MeOH, respectively. The combined crude extracts of *S. siliquastrum* were fractionated into *n*-hexane, 85% aq. MeOH, *n*-BuOH, and water fractions. The 85% aq. MeOH fraction was subjected to C_{18} reversed-phase vacuum flash chromatography using sequential mixtures of MeOH and water as eluents, followed by reversed-phase HPLC to yield six chromanols including new three chromanols (Fig. 1). The three known chromanols were readily identified as two diastereomeric sargachromanols D (**1**) and E (**2**) and sargachromanol K (**3**) by a combination of spectroscopic analysis and comparison with data reported for these compounds.[24)]

Two closely related metabolites, **4** and **5**, were isolated as a colorless gum. They had the same molecular formula $C_{27}H_{42}O_5$ determined by combined high resolution (HR)-FAB mass and ^{13}C-NMR spectrometry, and were diastereomeric to each other at C-9′ or C-10′ as **1** and **2**. The NMR spectral data of the former were very similar to those obtained for sargachromanol D (**1**) while those of the latter were very close to those derived from sargachromanol E (**2**). The only significant differences in the ^{13}C-NMR spectrum

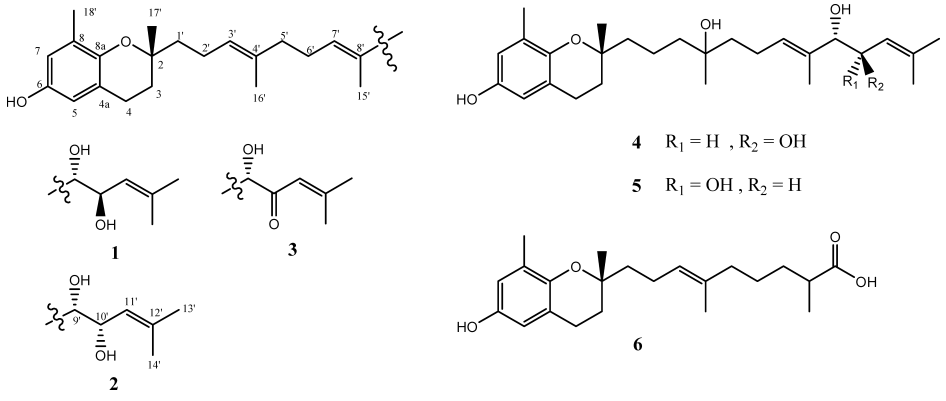

Fig. 1. Chemical Structure of Compounds **1**—**6** Isolated from *Sargassum siliquastrum*

* To whom correspondence should be addressed. e-mail: ywseo@hhu.ac.kr

were replacements of two olefinic signals at C-3′ and C-4′ olefinic carbons of **1** and **2** with methylene carbon signals at δ 43 and oxygen-bearing quaternary carbon signals at δ 73 of **4** and **5**. Corresponding changes were also observed in the ^{1}H-NMR spectrum in which the two olefinic proton signals[24] disappeared at δ 5.17 (1H, t, J=6.5) and 5.15 (1H, t, J=6.7) of **1** and **2**, respectively. These differences were explained by a hydration of C-3′ double bond of **1** and **2**. The location of the hydroxy group was assigned to C-4′ by a combination of gDQCOSY, TOCSY, gHMQC, and gHMBC experiments. Particularly, gHMBC correlations of an oxygen-bearing quaternary carbon (δ 73) with neighboring protons, including the methyl protons at δ 1.17 and 1.14 of **4** and **5**, respectively, were very helpful to define it. Thus, the structure of **4** was determined as 13-(3,4-dihydro-6-hydroxy-2,8-dimethy-2*H*-1-benzopyran-2-yl)-2,6,10-trimethyl-trideca-(2*E*,6*E*)-diene-4,5,10-triol.

In addition to the common asymmetric centers of the chromanols, **4** and **5** possess three additional asymmetric centers at C-4′, C-9′ and C-10′ of linear prenyl part. Relative configurations of C-9′ and C-10′ were assigned as 9*S** and 10*R** for **4** and as 9*S** and 10*S** for **5**, respectively, based on comparison of NMR data with structurally similar compounds **1** and **2**. (Tables 2, 3)[24] However, due to the limited amount of material available, the stereochemistry at C-7′ could not be assigned definitively.

An analogous metabolite **6** was isolated as a colorless gum. The molecular formula $C_{22}H_{32}O_4$ was deduced by a combination of high-resolution mass and ^{13}C-NMR spectral data. This compound was highly compatible with those of sargachromanol D (**1**). However, there were significant differences in the ^{13}C-NMR spectrum. The most noticeable difference was the absence of an isoprene unit at the terminal part of the linear prenyl chain. The presence of the carboxylic acid group was also readily recognized by carbon signal at δ 181 and strong absorption band at 1680 cm^{-1} in IR spectrum.

gHMBC long-range correlations of the methyl protons at δ 1.57 and 1.17 with neighboring carbons, combined with ^{1}H–^{1}H gDQCOSY and TOCSY of the olefinic and methyl protons with upfield protons, defined structure of the linear triprenyl portion. Carboxylic acid group was located at the terminus (C-9′) of the prenyl chain on the basis of long-range correlation between carboxylic carbon and neighboring protons. Thus, the structure of this compound was defined as 9-(3,4-dihydro-6-hydroxy-2,8-dimethy-2*H*-1-benzopyran-2-yl)-2,6-dimethyl-(6*E*)-nonenoic acid.

The antioxidant effect of the isolated compounds was investigated on human fibrosarcoma HT 1080 cells. In order to avoid cytotoxic interference of these compounds at high concentrations, the influence of the sample on cell viability of HT 1080 cells was determined using 3-(4,5-dimethylthiazol-2-yl)-5-diphenyltetrazolium bromide (MTT) assay.[31] All examined compounds did not exhibit any cytotoxicity in HT 1080 cells at concentration of 5 μg/ml during 1 h incubation (Fig. 2). Therefore, each of these compounds was used for the antioxidant experiments at concentration of 5 μg/ml.

The intracellular radical scavenging effect of isolated compounds was assessed using the 2′,7′-dichlorodihydrofluorescein deacetate (DCF-DA) fluorescence dye.[32] DCF-DA crosses cell membranes and is hydrolyzed enzymatically by intracellular esterase to nonfluorescent DCFH. Generation of

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for Compounds **4**—**6**

No.	**4**[a]		**5**[b]		**6**[a]	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2		75.4 s		76.3 s		75.3 s
3	1.74 (2H, m)	31.4 t	1.75 (2H, m)	32.7 t	1.77 (2H, m)	31.4 t
4	2.67 (2H, t, 6.3)	22.6 t*	2.67 (2H, t, 6.7)	23.5 t	2.69 (2H, t, 6.7)	22.6 t
4a		121.1s		122.1 s		121.2 s
5	6.35 (1H, d, 2.5)	112.5 d	6.30 (1H, d, 2.5)	113.4 d	6.36 (1H, d, 3.0)	112.5 d
6		147.8 s		150.1 s		147.6 s
7	6.44 (1H, d, 2.5)	115.6 d	6.38 (1H, d, 2.5)	116.5 d	6.46 (1H, d, 3.0)	115.6 d
8		127.1 s		127.6 s		127.3 s
8a		145.6 s		146.2 s		145.9 s
1′	1.53 (2H, m)	40.1 t	1.53 (2H, m)	41.1 t	1.60 (2H, m)	39.7 t
2′	1.44 (2H, m)	18.7 t	1.47 (2H, m)	19.2 t	2.09 (2H, q, 8.0)	22.2 t
3′	1.44 (2H, m)	42.6 t	1.45 (2H, m)	43.1 t	5.11 (1H, tq, 7.0, 1.4)	124.5 d
4′		72.9 s		73.4 s		134.6 s
5′	1.53 (2H, t, 7.8)	40.8 t	1.44 (2H, m)	42.0 t	1.96 (2H, t, 7.0)	39.4 t
6′	2.13 (2H, q, 7.8)	22.6 t*	2.01 (2H, q, 7.2)	23.3 t	1.42 (2H, m)	25.4 t
7′	5.50 (1H, t, 6.9)	129.7 d	5.35 (1H, t, 7.2)	129.7 d	1.63 (2H, m)	33.2 t
8′		133.2 s		135.4 s	2.46 (1H, m)	39.1 d
9′	3.86 (1H, d, 6.8)	80.1 d	3.73 (1H, d, 8.0)	82.6 d		181.2 s
10′	4.31 (1H, dd, 8.9, 6.8)	69.3 d	4.22 (1H, dd, 9.3, 8.0)	71.3 d		
11′	5.18 (1H, dt, 8.9, 1.5)	123.4 d	5.04 (1H, br d, 9.3)	125.7 d		
12′		139.2 s		136.6 s		
13′	1.77 (3H, s)	26.2 q	1.68 (3H, d, 1.5)	26.2 q		
14′	1.73 (3H, s)	18.7 q	1.66 (3H, d, 1.5)	18.6 q		
15′	1.67 (3H, s)	11.9 q	1.57 (3H, s)	12.3 q	1.17 (3H, d, 6.9)	17.0 q
16′	1.17 (3H, s)	26.8 q	1.14 (3H, s)	27.0 q	1.57 (3H, s)	15.8 q
17′	1.23 (3H, s)	24.3 q	1.24 (3H, s)	24.6 q	1.25 (3H, s)	24.1 q
18′	2.10 (3H, s)	16.2 q	2.06 (3H, s)	16.5 q	2.13 (3H, s)	16.2 q

a, *b*) Measured in $CDCl_3$ and CD_3OD at 300 and 75 MHz, respectively. Assignments were aided by ^{1}H gDQCOSY, TOCSY, DEPT, gHMQC, and gHMBC experiments.

Table 2. Comparison of NMR Spectral Data for Compound **4** with Those for **1**

No.	**4**		**1**	
	δ_H (H, m, Hz)	δ_C	δ_H (H, m, Hz)	δ_C
6′	2.13 (2H, q, 7.8)	22.6 t	2.16 (2H, m)	26.0 t
7′	5.50 (1H, t, 6.9)	129.7 d	5.46 (1H, t, 7.3)	129.5 d
8′		133.2 s		133.5 s
9′	3.86 (1H, d, 6.8)	80.1 d	3.86 (1H, d, 6.6)	80.2 d
10′	4.31 (1H, dd, 8.9, 6.8)	69.3 d	4.30 (1H, dd, 8.8, 6.6)	69.2 d
11′	5.18 (1H, dq, 8.9, 1.5)	123.4 d	5.19 (1H, dq, 8.8, 1.5)	123.3 d
12′		139.2 s		139.2 s
13′	1.77 (3H, s)	26.2 q	1.77 (3H, s)	26.0 q
14′	1.73 (3H, s)	18.7 q	1.73 (3H, s)	18.6 q
15′	1.67 (3H, s)	11.9 q	1.65 (3H, s)	11.8 q

Measured in $CDCl_3$ at 300 and 75 MHz, respectively.

Table 3. Comparison of NMR Spectral Data for Compound **5** with Those for **2**

No.	**5**		**2**	
	δ_H (H, m, Hz)	δ_C	δ_H (H, m, Hz)	δ_C
6′	2.01 (2H, dt, 6.6, 7.2)	23.3 t	2.08 (2H, dt, 6.8, 7.3)	27.2 t
7′	5.35 (1H, t, 6.6)	129.7 d	5.34 (1H, t, 6.8)	129.4 d
8′		135.4 s		135.6 s
9′	3.73 (1H, d, 8.0)	82.6 d	3.71 (1H, d, 7.8)	82.7 d
10′	4.22 (1H, dd, 9.3, 8.0)	71.3 d	4.22 (1H, dd, 9.3, 7.8)	71.4 d
11′	5.04 (1H, br d, 9.3)	125.7 d	5.03 (1H, br d, 9.3)	125.8 d
12′		136.6 s		136.9 s
13′	1.68 (3H, d, 1.0)	26.2 q	1.67 (3H, d, 1.0)	26.1 q
14′	1.66 (3H, d, 1.0)	18.6 q	1.66 (3H, d, 1.0)	18.5 q
15′	1.57 (3H, s)	12.3 q	1.57 (3H, s)	12.3 q

Measured in CD_3OD at 300 and 75 MHz, respectively.

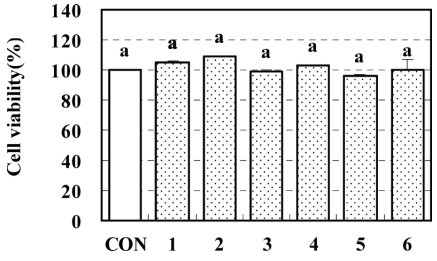

Fig. 2. Cytotoxic Effects of the Compounds in HT 1080 Cells Using MTT Assay

Cells were treated with 5 μg/ml concentration of the compounds for 24 h.

intracellular ROS such as H_2O_2 and hydroxyl radical oxidizes DCFH to highly fluorescent DCF in cells.[33] As a result, the intensity of the fluorescence emitted by DCF shows a radical scavenging effect of the tested compounds. As shown in Fig. 3, all compounds exhibited more than a 67.2% decrease in generation of ROS at concentrations of 5 μg/ml, compared to the control. In particular, compound **2** showed the strongest scavenging effect with an 87.2% decrease of ROS generated in HT 1080 cells.

When excessive ROS is generated, free radicals react strongly with unsaturated lipids and cause lipid peroxidation, which decreases cell membrane fluidity and induces cell death. Therefore, determining the degree of lipid peroxidation and antioxidant activity is significantly important. The

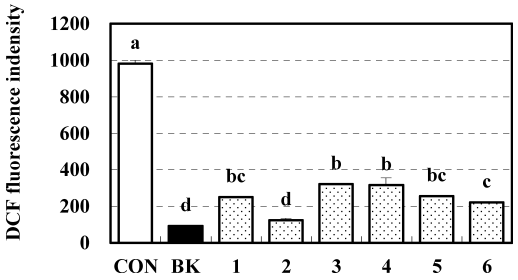

Fig. 3. Inhibitory Effects of the Compounds on Intracellular Generation of ROS at 5 μg/ml

Intracellular formation of ROS was assessed using oxidation sensitive dye, DCFH-DA. [a—d] Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

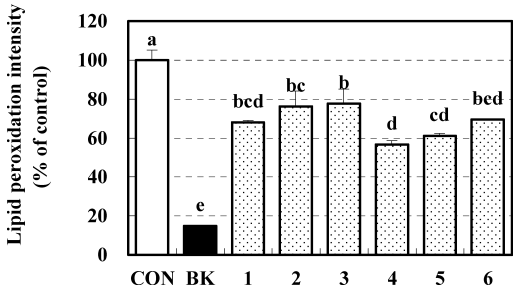

Fig. 4. Effect of Seaweed Extracts on Membrane Lipid Peroxidation at 50 μg/ml

Membrane lipid peroxidation determined by TBARS method. [a—e] Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

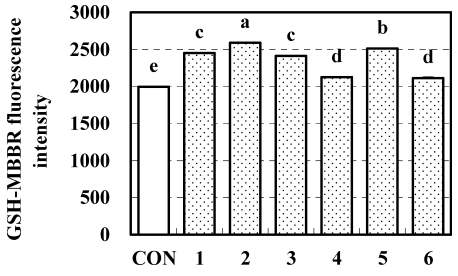

Fig. 5. Effects of the Compounds on Regulation of GSH Level in HT 1080 Cells at 5 μg/ml

[a—e] Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

antioxidant effect of these compounds on lipid peroxidation was measured with the thiobarbituric acid-reactive substance (TBARS) assay.[33] As shown in Fig. 4, lipid peroxidation was decreased in all compounds, and compounds **4** and **5** showed relatively good scavenging rates of 43.2% and 38.9%, respectively, at the concentration of 50 μg/ml.

GSH is the most abundant low molecular weight thiol inside mammalian cells, and changes in glutathione level directly reflect intracellular redox alterations.[34] To investigate the relationship between the increased ROS and the level of antioxidant materials in cells, the intracellular GSH level was measured.[34,35] Intracellular GSH levels were significantly increased in the presence of each compound, compared with those in the absence of compounds as shown in Fig. 5. Compounds **1**—**3** and **5** showed higher intracellular GSH levels than those of compounds **4** and **6**.

In conclusion, six chromanols were isolated from *S. sili-*

quastrum, and their structures were elucidated based on 2D-NMR and MS spectrometry. Not only did all compounds effectively inhibit intracellular ROS formation and lipid peroxidation induced by H_2O_2, but also they increased intracellular GSH level. Further investigations on the bioactivities of these compounds are currently in progress.

Experimental

General Experimental Procedures Optical rotation was determined on a Perkin-Elmer polarimeter 341 using a 1 cm cell. NMR spectra were recorded in CD_3OD and $CDCl_3$ on a Varian Mercury 300 instrument at 300 MHz for ^1H and 75 MHz for ^{13}C, respectively, using standard pulse sequence programs. All chemical shifts were recorded with respect to tetramethylsilane (TMS) as an internal standard. Mass spectral data were obtained at the Korean Basic Science Institute, Seoul, Korea. High performance liquid chromatography (HPLC) was performed with a Dionex P580 pump system equipped with Varian 350 RI detector. All solvents used were spectroscopic grade or were distilled from glass prior to use.

Plant Material The brown alga *Sargassum siliquastrum* was collected along the off shore of Jeju Island, Korea in May, 2003. The sample was identified by Dr. Jong Soo Yoo by its morphological character.

Extraction and Isolation The freshly-collected samples were frozen and remained frozen until chemically investigated. The samples were defrosted and repeatedly extracted for 2 d with a mixture (1 : 1) of acetone–CH_2Cl_2 (1.5 l×2) and MeOH (1.5 l×2), respectively. The combined crude extracts (57.25 g) were evaporated under reduced pressure and then partitioned between CH_2Cl_2 and water. The organic layer was further partitioned between 85% aq. MeOH (8.05 g) and *n*-hexane (7.24 g), and the aqueous layer was fractionated with *n*-BuOH (3.02 g) and H_2O (38.94 g). The 85% aqueous MeOH fraction was subjected to C_{18} reversed-phase vacuum flash chromatography using stepwise gradient mixtures of MeOH and water (50, 60, 70, 80, 90% aq. MeOH, and 100% MeOH), and finally 100% ethyl acetate as eluent to give 7 subfractions. The fifth fraction (0.24 g) was subjected to reversed-phase HPLC (YMC ODS-A, 87% aq. MeOH, 1 cm×25 cm, 2 ml/min) to yield 34 subfractions. Subfractions 16 and 20 were identified as compound **1** (9.7 mg) and **2** (23.0 mg), respectively. Subfractions 17 (23.6 mg) (2.0 mg) and 24 (22.0 mg) were further separated with reversed-phase HPLC (YMC ODS-A, 75% aq. MeCN, 1 cm×25 cm, 2 ml/min) to yield compound **6** (2.0 mg) and **3** (5.0 mg), respectively. The fourth fraction (0.27 g) was subjected to reversed-phase HPLC (YMC ODS-A, 76% aq. MeOH, 1 cm×25 cm, S-5 μm, 2 ml/min) to yield compounds **4** (6.0 mg) and **5** (7.2 mg).

Compound **4**: Colorless gum; $[\alpha]_D^{25}$ +7.50° (c=0.13, MeOH); Positive HR-FAB-MS m/z 446.3066 $[M]^+$ (Calcd for $C_{27}H_{42}O_5$, 446.3032); ^{1}H- and ^{13}C-NMR , see Tables 1 and 2.

Compound **5**: Colorless gum; $[\alpha]_D^{25}$ +23.33° (c=0.13, MeOH); Positive HR-FAB-MS m/z 446.3066 $[M]^+$ (Calcd for $C_{27}H_{42}O_5$, 446.3032); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Compound **6**: Colorless gum; $[\alpha]_D^{25}$ +12.00° (c=0.17, MeOH); IR (NaCl) cm^{-1}: 3400—3300, 1680; high resolution electron impact (HR-EI)-MS m/z 360.2327 $[M]^+$ (Calcd for $C_{22}H_{32}O_4$, 360.2301); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Cell Culture Human fibrosarcoma HT1080 cells were grown at 5% CO_2 and 37 °C in a humidified atmosphere using Dulbecco's modified Eagle's medium (DMEM, Gibco Co., U.S.A.) supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine and 100 μg/ml penicillin–streptomycin (Gibco Co., U.S.A.). The medium was replaced every 2—3 d.

Cell Cytotoxicity Cytotoxic levels of the compounds on cultured cells were measured using MTT assay,[31] which is based on the conversion of MTT to MTT-formazan by mitochondrial enzyme. The cells were seeded onto 96-well microplates at a density of 5×10^3 cells/well for 24 h. Then the cells were treated with control medium or the medium supplemented with 5 μg/ml compounds. After incubation of 1 h, 100 μl of MTT solution (1 mg/ml) was added and incubated for 4 h. Finally, 150 μl of dimethyl sulfoxide (DMSO) was added to solubilize the formed formazan crystals, and the amount of formazan crystal was determined by measuring the absorbance at 540 nm using a multidetection microplate fluorescene spectrophotometer synergy HT (Bio-Tek Instrucments Inc., Winooski, VT, U.S.A.). Relative cell viability was determined by the amount of MTT converted into formazan crystal. Viability of cells was quantified as a percentage compared to the control, and dose response curves were developed.

Determination of Intracellular Formation of ROS Using DCF-DA Labeling Intracelluar formation of ROS was assessed using oxidation sensitive dye DCF-DA as the substrate.[32] HT 1080 cells growing in fluorescence microtiter 96-well plated were loaded with 0.02 mM DCF-DA in Hank's balanced salt solution (HBSS) and incubated for 20 min in the dark. Cells were then treated with 5 μg/ml compounds and incubated for 1 h. After the cells were washed with phosphate buffered saline (PBS) 3 times, 500 μM H_2O_2 dissolved in HBSS was added to the cells. The fluorescence of DCF was detected after 120 min at the excitation wavelength (Ex) of 485 nm and the emission wavelength (Em) of 528 nm using a multidetection microplate fluorescene spectrophotometer synergy HT (Bio-Tek Instruments Inc.). Effects of the compounds were plotted and compared with fluorescence intensity of control and blank groups.

Membrane Lipid Peroxidation Assessment by TBARS Method The inhibitory effect on lipid peroxidation was measured spectroscopically by the TBARS method[33] with some modification. In brief, 200 μl of PBS-suspended HT 1080 cells in 1.5 ml e-tube was incubated for 10 min with test samples and distilled water as a control. One hubndred microliters of H_2O_2 (2 mM H_2O_2) and 100 μl of $FeSO_4$ (0.1 M $FeSO_4$) were added to cells, and then incubated at 37 °C for 10 min. The oxidation reaction was terminated by adding 50 μl of cold trichloroacetic acid (TCA, 20% w/v). Four hundred and fifty microliters of TBA (1% w/v) was added to the content tube and then the mixture was heated at 90 °C for 30 min. After cooling, the flocculent precipitate was removed by centrifugation and the absorbance of the supernatant was measured at 528 nm.

Measurement of Intracellular GSH The intracellular glutathione (GSH) level in intact cells was determined using monobromobimane as a thiol-staining reagent.[34] HT1080 cells were seeded into fluorescence 96-well plates at a density of 5×10^3 cells/well. Cells were treated with 5 μg/ml of the compounds and incubated for 30 min. Monobromobimane dissolved in 1% DMSO was added to cells at a final concentration of 0.04 mM and staining was carried out for 120 min at 37 °C in the dark. After staining fluorescence intensity was measured (excitation and emission: 360 and 465 nm) using a multidetection microplate fluorescene spectrophotometer synergy HT (Bio-Tek Instruments Inc.). The averaged fluorescence values of cell populations were plotted and compared with the control group in which cells were grown without treatment of the compounds.

Statistical Analysis Data were analyzed using the analysis of variance (ANOVA) procedure of Statistical Analysis System (SAS Institute 1999—2001). Significant differences between treatment means were determined by using Duncan's multiple range tests. Significance of differences was defined at the $p<0.05$ level.

Acknowledgement This research was financially supported by a Grant (No. 70008305) from the Ministry of Knowledge Economy (MKE) and Korea Institute for Advancement of Technology (KIAT) through the Research and Development for Regional Industry. Mass spectral data were kindly provided by Korea Basic Science Institute.

References

1) Nanba A., Okuda T., *Nippon Suisan Gakkaishi*, **59**, 1675—1678 (1993).
2) Numata A., Kanabara S., Takahashi C., Fujiki R., Yoneda M., Fujita E., Nabeshima Y., *Chem. Pharm. Bull.*, **39**, 2129—2131 (1991).
3) Perez-Castorena A. L., Arciniegas A., Apan M. T. R., Villasenor J. L., de Vivar A. R., *Planta Med.*, **68**, 645—647 (2002).
4) Seo Y., Lee H.-J., Park K. E., Kim Y. A., Ahn J. W., Yoo J. S., Lee B.-J., *Biotechnol. Bioprocess Eng.*, **9**, 212—216 (2004).
5) Iwashima M., Mori J., Ting X., Matsunaga T., Hayashi K., Shinoda D., Saito H., Sankawa U., Hayashi T., *Biol. Pharm. Bull.*, **28**, 374—377 (2005).
6) Tsang C. K., Ina A., Goto T., Kamei Y., *Neuroscience*, **132**, 633—643 (2005).
7) Seo Y., Park K. E., Nam T. J., *Bull. Kor. Chem. Soc.*, **28**, 1831—1833 (2007).
8) Park B.-G., Kwon S.-C., Park G.-M., Ham J., Shin W.-S., Lee S., *Bioorg. Med. Chem. Lett.*, **18**, 6324—6326 (2008).
9) Kim Y. A., Kong C. S., Um Y. R., Lee J. I., Nam T. J., Seo Y., *Ocean Science Journal*, **43**, 31—37 (2008).
10) Kusumi T., Shibata Y., Ishitsuka M., Kinoshita T., Kakisawa H., *Chem. Lett.*, **1979**, 277—278 (1979).
11) Ishitsuka M., Kusumi T., Nomura Y., Konno T., Kakisawa H., *Chem. Lett.*, **1979**, 1269—1272 (1979).
12) Nakayama M., Fukuoka Y., Nozaki H., Matsuo A., Hayashi S., *Chem. Lett.*, **1980**, 1243—1246 (1980).

13) Kikuchi T., Mori Y., Yokoi T., Nakazawa S., Kuroda H., Masada Y., Kitamura K., Kuriyama K., *Chem. Pharm. Bull.*, **31**, 106—113 (1983).
14) Segawa M., Shirahama H., *Chem. Lett.*, **1987**, 1365—1366 (1987).
15) Amico V., Neri P., Oriente G., Piattelli M., *Phytochemistry*, **28**, 215—219 (1989).
16) Numata A., Kanabara S., Takahashi C., Fujiki R., Yoneda M., Usami Y., Fujita E., *Phytochemistry*, **31**, 1209—1213 (1992).
17) Rivera P., Podesta F., Norte M., Cataldo F., Gonzalez A. G., *Can. J. Chem.*, **68**, 1399—1400 (1990).
18) Davyt D., Enz W., Manta E., Navarro G., Norte N., *Nat. Prod. Lett.*, **9**, 305—312 (1997).
19) Rivera P., *Bol. Soc. Chil. Quim.*, **41**,103—105 (1996).
20) Mesguiche V., Valls R., Piovetti L., Banaigs B., *Phytochemistry*, **45**, 1489—1494 (1997).
21) Urones J. G., Basabe P., Marcos I. S., Pineda J., Lithgow A. M., Moro R. F., Brito Palma F. M. S., Araujo M. E. M., Gravalos M. D. G., *Phytochemistry*, **31**, 179—182 (1992).
22) Urones J. G., Araujo M. E. M., Brito Palma F. M. S., Basabe P., Marcos I. S., Moro R. F., Lithgow A. M., Pineda J., *Phytochemistry*, **31**, 2105—2109 (1992).
23) Seo Y., Park K. E., Kim Y. A., Lee H.-J., Yoo J.-S., Ahn J.-W., Lee B.-J., *Chem. Pharm. Bull.*, **54**, 1730—1733 (2006).
24) Jang K. H., Lee B. H., Choi B. W., Lee H.-S., Shin J., *J. Nat. Prod.*, **68**, 716—723 (2005).
25) Jung M., Jang K. H., Kim B., Lee B. H., Choi B. W., Oh K.-B., Shin J., *J. Nat. Prod.*, **71**, 1714—1719 (2008).
26) Cho S. H., Choi J. Y., Kang S. E., Hong Y. K., Ahn D. H., *J. Environ. Biol.*, **29**, 479—484 (2008).
27) Heo S.-J., Jeon Y.-J., *J. Photochem. Photobiol. B*, **95**, 101—107 (2009).
28) Lim S. N., Cheung P. C. K., Ooi V. E. C., Ang P. O., *J. Agric. Food Chem.*, **50**, 3562—3866 (2002).
29) Cho S. H., Kang S. E., Cho J. Y., Kim A. R. , Park S. M., Hong Y. K., Ahn D. H., *J. Med. Food*, **10**, 479—485 (2007).
30) Park B. G., Kwon S. C., Park G. M., Ham J., Shin W. S., Lee S., *Bioorg. Med. Chem. Lett.*, **24**, 6324—6326 (2008).
31) Hansen M. B., Nielsen S. E., Berg K., *J. Immunol. Meth.*, **119**, 203—210 (1989).
32) LeBel C. P., Ischiopoulos H., Bondy S. C., *Chem. Res. Toxicol.*, **5**, 227—231 (1992).
33) Heath R. L., Packer L., *Arch. Biochem. Biophys.*, **125**, 189—198 (1968).
34) Schafer F. Q., Buettner G. R., *Free Radic. Biol. Med.*, **30**, 1191—1212 (2001).
35) Elbekai R. H., EI-Kai A. O. S., *Free Radic. Biol. Med.*, **39**, 1499—1511 (2005).